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Mark B. Wilson

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Philip G. ASHTON-RICKARDT and
Joseph T. OPFERMAN

Serial No.: 09/993,363

Filed: Nov. 14, 2001

For: INDUCTION OF IMMUNITY USING INHIBITORS OF GRANZYMES

Group Art Unit: 1632

Examiner: R. R. Shukla

Atty. Dkt. No.: ARCD:382US

DECLARATION OF RAYMOND M. WELSH, PH. D., UNDER 37 C.F.R. § 1.132

Hon. Commissioner for Patents
Washington, D.C. 20231

I, Raymond M. Welsh, Ph.D., do declare that:

1. I am a United States citizen residing at 76 South Quinsigamond Ave. Unit 4, Shrewsbury, Massachusetts, 01545.

2. I currently hold the position of Professor, Department of Pathology, University of Massachusetts Medical Center (Worcester, MA). A copy of my NIH Biographical Sketch is attached as Appendix A, and a copy of my curriculum vitae is attached as Appendix B. Appendix B includes a numbered list of my publications.

3. I am a skilled virologist, who understands the immunology of viral infection, as evidenced by the following:

- I have worked with LCMV for over thirty years (since 1969), and I have collaborated and published with Nobel Lauriat Rolf Zinkernagel, whose work pertaining to LCMV is quoted and presented below.
- I have expertise in HIV, having served on the State of California AIDS task force for about ten years.
- I am Editor for viral immunology and pathogenesis articles for the Journal of Virology, and am in charge of the review of many of the papers on the immune response to HIV, hepatitis virus, CMV, and other viruses.
- I study and have NIH grants on the topic of apoptosis of T cells and on T cell memory.
- I published some of the first work on LCMV-induced T cells having enzyme-containing granules (reference #102 in Appendix B) and in documenting apoptosis as a regulator of T cell responses during LCMV infection (*e.g.*, references #121 and 135 in Appendix B).
- In addition, I have just published a paper in the journal, *Immunity*, on T cell apoptosis in the LCMV system and in analyzing granzyme mRNA levels within these T cells.

4. I am being compensated for my time in preparing this declaration, but not for the content of my testimony.

5. I have reviewed the above-referenced application, as well as the Office Action to the above-referenced application that is dated March 19, 2003. I understand that the above-referenced application was filed on November 14, 2001.

6. I understand that the Examiner has rejected claims 26-50 of the above-referenced application because the Examiner believes that the claims contain subject matter which was not described in the specification in such a way as to enable a skilled virologist to make and/or use the invention. In addition, I understand that the Examiner believes that it would require undue experimentation for a skilled virologist to use the claimed invention as it is currently claimed. More particularly, I understand that the Examiner believes the specification does not teach inducing or enhancing immunity in a subject against human immunodeficiency virus (HIV), and that the Examiner questions whether the results disclosed in the specification, particularly those pertaining to transgenic mice and lymphocytic choriomeningitis virus (LCMV) infection, are applicable to HIV or any other virus.

7. A skilled virologist with an ordinary understanding of viral immunology would have recognized, at the time the above-referenced application was filed, that LCMV infection in mice is a model for determining the usefulness of the claimed invention for treating other viral diseases, including HIV. I believe that a skilled virologist with an ordinary understanding of viral immunology would have understood, at the time the above-referenced application was filed, that LCMV infection in mice was a model for HIV infection in humans.

8. My positions with respect to the accepted nature of the LCMV mouse model is supported by literature that would be familiar to one having an ordinary understanding of viral immunology. For example, the following papers provide facts in support of the correlation between LCMV infection and HIV infection:

Zinkernagel, Vaccine 20:1913-1917, 2002 (Exhibit 1):

On page 1914, it is noted that LCMV has contributed considerably in the past 10 years to a better understanding of HIV-AIDS pathogenesis (Table 1)." Table 1 (p. 1914) provides a summary of these contributions with references.

Klenerman and Zinkernagel, Immunological Reviews 159:5- 16, 1997 (Exhibit 2):

- Regarding the state of knowledge pertaining to LCMV infection in the mouse (page 5):

"[T]his infectious model has been established for over 60 years. The in vivo roles of specific immune subsets in the clearance of virus and the induction of disease are well understood. The mechanisms which allow particular virus strains to establish persistent infections have been dissected in fine detail, in particular with the use of transgenic and knockout mice."

- Regarding the rationale for comparing the LCMV mouse model to HIV (page 6):

"The reason for embarking on such a comparison is that the dominant immune response to both viruses is the cytotoxic T lymphocyte (CTL), and particular features of this immune response have striking parallels in the two infections. Since the CTL response to LCMV has been studied in immense detail, and its role *in vivo* has been accurately determined both qualitatively and quantitatively, it provides an excellent reference point from which to view the role of the same cellular response in HIV."

- An analysis of the similarities between LCMV infection and HIV infection and other viruses with regard to the role of CTL in virus infection is provided on pages 6-13. For instance, it is noted that:

  - Page 6: "The effects of CTL-mediated killing in LCMV and HIV are therefore, importantly, 2-fold: they kill infected cells and they reduce virus production."

  - Page 8: "The acute response and initial control phase in both LCMV and HIV is dominated by CD8-positive MHC class I-restricted CTL."

  - Page 9: "Such very high levels of CTL are by no means restricted in man to HIV, since similar responses occur in Epstein-Barr virus (EBV) during the onset of acute symptoms. Acute influenza also induces expansions of CTL with restricted TCR usage depending on HLA type of the individual, and, briefly, the capacity to kill directly *ex vivo*."

- Page 9: "Antiviral CTL remain at an elevated frequency for many months after initial LCMV infection. . . In HIV, a similar situation of active circulating CTL (Fig. 4B) and elevated precursor frequencies is seen, although, in this case, it is clear that continuous virus exposure is taking place."

- Page 10: "The loss of killing activity is central to the issue of CTL in control of viruses. The phenomenon of exhaustion was first demonstrated in LCMV, and has been proposed as a mechanism for CTL decline in HIV."

- Page 11: "The major issue in HIV is the long-term disappearance of CTL responses. . . Thus, CTL exhaustion in HIV may occur in the acute phase in an analogous manner to LCMV, or as a more chronic process in the long term. The requirements for exhaustion as defined in LCMV are extensive replication, rapid turnover of virus, infection of lymphohaemopoietic cells (plus probable extensive replication in peripheral organs) and a vigorous initial CTL response – HIV is well qualified in all these areas."

- Page 13: "Both [HIV and LCMV] induce a substantial CTL response which dominates the early stages of infection and probably determines the ultimate outcome."

Borrow *et al.*, J. Virology, 69:1059-1070, 1995 (Exhibit 3): This paper addresses the virus-induced immunosuppression induced by LCMV, the role of virus tropism in determining pathogenicity, the role of dendritic cells. Similarities of LCMV to HIV are discussed.

- Abstract: "Our findings illustrate the key role that virus tropism may play in determining pathogenicity and, further, document a mechanism for virus-inducted immunosuppression which may contribute to the clinically important immune suppression associated with many virus infections, including human immunodeficiency virus type I."
- Pages 1068-69: "Can our finding that virus infection of dendritic cells is a critical step in the production of immune suppression by LCMV clone 13 be generalized to other virus infections? It is of interest that all viruses known to be able to persist in vivo have been shown to infect cells of the immune system. . . In view of the central location of [dendritic cells] within the immune system and their unique, critical functions in the initiation of immune responses, it is likely that virus infection of dendritic cells and subsequent impairment of their functions will prove to be an underlying factor in many examples of generalized immune suppression associated with virus infection."

Ciurea *et al.*, Proc. Natl. Acad. Sci. USA, 96:11964-11969, 1999 (Exhibit 4): This paper discusses the persistence of LCMV at very low levels in the immune mouse, and compares the results to HIV infection and other viruses:

- Regarding similarities of LCMV to HIV and other viruses (abstract):

> "The finding that LCMV-WE persists in the face of apparently intact immune responses resembles the situation in some viral (hepatitis B and C, HIV) and bacterial (tuberculosis, leprosy) infections in humans; the results are relevant to the understanding not only of other murine and human persistent viral infections but also of protective immunological memory by 'infection immunity.'"

Odermatt *et al.*, Proc. Natl. Acad. Sci. USA, 88:8252-8256, 1991 (Exhibit 5): This paper shows that LCMV-induced acquired immune suppression in mice is caused by $CD8^+$-T-cell-dependent elimination of macrophages/antigen-presenting cells (abstract).

- A comparison to HIV is discussed on page 8254-8255:

> "A possible $CD8^+$ -T-cell-dependent pathogenesis of AIDS has been proposed to explain reduction of infected or HIV-antigen-binding $CD4^+$ T cells. It is conceivable that in analogy to the immunopathology observed during a LCMV infection, virus-specific cytotoxic T cells (and probably not the virus itself) may be responsible for both numerical and functional reduction of macrophages and antigen-presenting cells and thus cause destruction of follicular structures in HIV infections. Detailed histopathological studies may be taken to support the hypothesis of $CD8^+$-T-cell-dependent immunopathology may significantly contribute to the pathogenesis of AIDS; lymph node histopathology in patients with AIDS-related complex is often strikingly similar to that of mice suffering from LCMV-induced immunosuppression shown here."

9. Based on my review of these materials and my experience, a skilled virologist with an ordinary understanding of viral immunology would have understood, at the time the above-referenced application was filed, that LCMV infection in mice is a model for HIV infection in humans.

10. My review of the specification identified the following sections pertaining to LCMV infection and HIV infection:

- page 8, lines 11 –15: Indicates that the methods of the present invention provide enhanced immunity to a wide variety of viruses, including HIV and other viruses.
- page 10, lines 6-19: Indicates methods by which the present invention provides for methods for alleviating HIV infection.
- page 14, lines 6-17: Indicates that the present inventors have demonstrated the ability of granzyme B inhibitors to successfully eliminate virus, as shown using the transgenic mouse model of LCMV infection.
- page 50, line 10 through page 52, line 12; Example 1, page 67, line 22 through page 69, line 19: Provides information regarding selected experimental techniques, including production of transgenic animals, infection with LCMV, and CTL assays.
- Example 2, page 69, line 24 through page 71, line 29: Experimental results demonstrating that mouse Serpin SPI6 protects cells from apoptosis by granzyme B.
- Example 3, page 72, lines 4-31: Provides information regarding production of transgenic mice overexpressing SPI6 serpin.
- Example 4, page 74, line 5 through page 77, line 6: Results demonstrating that SPI6 protects cells from mouse granzyme B.
- Example 6, page 77, line 29 through page 78, line 10: Demonstrates that SPI6 enhances CTL activity and protects from apoptosis.
- Example 7, page 78, line 15 through page 79, line 2: Results demonstrating clonal exhaustion induced by LCMV in mice.

- Example 9, page 81, line 29 through page page 83, line 4: Results demonstrating the role of SPI6 in the control of transgenic TCR CTL function.
- Example 12, page 84, line 10 through page 88, line 25: LCMV studies demonstrating that granzyme B is involved in the development of memory cells.

11. Based on my review of these sections of the specification and in view of the literature pertaining to the usefulness of LCMV infection as a model for HIV infection, the present claims contain subject matter which was described in the specification in such a way as to enable a skilled virologist with an ordinary understanding of viral immunology to make and/or use the invention.

12. Further, in view of the above, no undue experimentation would be required for a skilled virologist with an ordinary understanding of viral immunology to make and/or use the claimed invention of the above-referenced application as it is currently claimed.

13. In view of the above, a skilled virologist with an ordinary understanding of viral immunology would have understood, at the time the above-referenced application was filed, that the specification teaches inducing or enhancing immunity in a subject against human immunodeficiency virus.

14. Additionally, in view of the above, LCMV infection is a model for HIV infection that is accepted by those who work in virology and viral immunology.

15. I hereby declare that all statements made herein of my knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of

the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

June 16, 2003
Date

Raymond M. Welsh, Ph.D.